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Plasminogen Activator Inhibitor (PAI-1) Antigen Levels in Primary TTP and Secondary TTP Post-Bone Marrow Transplantation

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Our objectives were to measure and compare plasminogen activator inhibitor levels (PAI-1) in primary adult thrombotic thrombocytopenic purpura (TTP) and in secondary TTP associated with bone marrow transplantation (BMT)-TTP. PAI-1 antigen levels were measured by an enzyme linked immunosorbent assay on platelet poor plasma samples obtained from patients at the time of diagnosis of the TTP disorder and from a group of normal volunteers. The samples were frozen at −70°C. Patients with TTP secondary to bone marrow transplantation had their grade determined by percentage fragmented cells and lactate dehydrogenase levels. The primary TTP samples were contributed by investigators in the multi-institutional North American TTP Group, and the bone marrow transplant samples were obtained from an adult bone marrow transplant program. Nineteen patients with adult TTP, and 47 patients with bone marrow transplant-TTP were evaluated. Of the latter, 14 had Grade 2, 13 had Grade 3, and 20 had Grade 4 BMT-TTP. PAI-1 levels were elevated compared to control volunteers in both primary adult TTP and BMT-TTP, $P < 0.001$. Levels did not differ from normal in Grade 2 BMT-TTP (median = 16 ng/ml; quartiles = 9–20). PAI-1 levels were similar in primary TTP (median = 32 ng/ml; quartiles = 25–51) and Grade 3 BMT-TTP (median = 35 ng/ml; quartiles = 19–48 ng/ml), $P = 0.7$. However, PAI-1 levels were significantly higher in Grade 4 BMT-TTP (median = 83 ng/ml; quartiles = 60–143) than Grade 3 BMT-TTP, and primary TTP, $P < 0.001$. PAI-1 levels are high in primary TTP and secondary bone marrow transplant-TTP (Grades 3–4). In contrast, normal levels are seen in Grade 2 BMT-TTP, which is a self-limited disorder. Therefore, high PAI-1 levels may contribute to hypofibrinolysis in the pathogenesis of primary TTP and of moderate to severe TTP (Grades 3–4) following bone marrow transplantation. Am. J. Hematol. 59:9–14, 1998. **© 1998 Wiley-Liss, Inc.**

Key words: TTP; plasminogen activator inhibitor; TTP post bone marrow transplantation

INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are disorders characterized by the presence of microangiopathic hemolytic anemia and thrombocytopenia [1]. Typically TTP occurs in adults and characteristic clinical features include neurologic defects, renal dysfunction, and fever [2]. Untreated patients with TTP usually die; plasmapheresis has had a great impact upon improving outcome in adult TTP [3].

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In contrast, HUS occurs in children, is associated with prominent renal dysfunction, and demonstrates little in the way of neurologic symptoms [4]. It is often a self-limited disorder and diarrhea-associated HUS has been associated with verocytotoxins [4]. A secondary TTP occurs following bone marrow transplantation [5–9]. It ranges from a self-limited to a lethal disorder and does not respond to exchange with plasma replacement [8–10].

Plasminogen activator inhibitor (PAI-1) is a glycoprotein that is normally present in plasma at low concentrations [11]. It exists in an active and an inactive form [11]. Active PAI-1 is produced by endothelial cells and hepatocytes and inactive PAI-1 may be released from platelets [12]. High levels of PAI-1 may contribute to a hypofibrinolytic state by complexing with tPA and/or urokinase, thereby decreasing the generation of plasmin [11]. Theoretically, hypofibrinolysis may worsen a microangiopathic disorder by allowing for accumulation of fibrin upon the platelet microthrombi within the microvasculature.

PAI-1 antigen levels have been shown to be elevated in patients with hemolytic uremic syndrome [13–16] and in adult TTP [17]. PAI antigen levels have not been reported in the secondary TTP following bone marrow transplantation. Therefore, the aim of this study was to measure PAI-1 antigen levels at the time of diagnosis in a series of patients with primary TTP and with secondary BMT-TTP.

METHODS

Excess citrated platelet-poor plasma that had been collected at diagnosis was used for these measurements. These blood samples were obtained from the patients following informed consent prior to participation in two clinical protocol studies. Both the patient and control samples had been frozen at −70°C prior to analysis.

Nineteen patients were participants in the North American TTP Study Group (NATG). This is a multi-institutional group that is conducting a trial to compare the effect of apheresis using either whole plasma or the cryosupernatant portion of plasma for replacement. All NATG patients gave informed consent for participation in this clinical study, which is approved by the individual participating institutional review boards. The TTP syndrome was unrelated to pregnancy, malignancy, chemotherapy, HIV disease, or transplantation.

The remaining patients were bone marrow transplant patients enrolled in a prospective study of bone marrow transplant associated TTP at the Adult Bone Marrow Transplant Program of the Western Pennsylvania Cancer Institute. All bone marrow transplant patients gave informed consent. This protocol was approved by the Institutional Review Board of the Western Pennsylvania Hospital.

The control group consisted of 41 healthy adult volunteers (19 males and 22 females, aged 25–55) whose citrated platelet-poor plasma was collected and frozen at −70°C for the same assays. The samples from the controls were handled in the same manner as the samples from the patients. The females were not pregnant and were not taking oral contraceptives.

Plasminogen Activator Inhibitor-1 Antigen (PAI-1) Assay

PAI-1 antigen was quantitated by an enzyme-linked immunosorbent assay using a sandwich technique (Diagnostica Stago, Asnieres-sur-Seine, France) [18]. The intra-assay and inter-assay coefficients of variation were 2.8% and 12.5%, respectively.

GVHD Prophylaxis

Cyclosporine A (CsA) and methylprednisolone were used as GVHD prophylaxis. In unrelated donor transplants, methotrexate was also given in doses of 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3 and +6 and +11. Patients received CsA at 2.5 mg/kg every 12 hr IV from day −1 to discharge. Post-discharge, CsA was administered at 7.5 mg/kg po bid and tapered to 0.5 mg/kg po bid at day 354 and discontinued at day 360 post-BMT. CsA doses were adjusted to maintain whole blood levels between 350–600 mcg/l, as measured by the polyclonal fluorescence polarization assay [19].

Corticosteroids were administered according to the following schedule: Methylprednisolone 0.25 mg/kg IV every 12 hr from 7–14 days post-BMT, 0.5 mg/kg IV every 12 hr from day 15–28, then oral Prednisone 0.4 mg/kg twice daily from day 29–42, 0.25 mg/kg twice daily from day 43–56, 0.1 mg/kg twice daily from day 57–119, and 0.1 mg/kg daily from day 120–180.

Preparative Regimens

Three preparative regimens were employed for bone marrow transplantation. Oral busulfan (Bu) 4 mg/kg/day for 4 days followed by 60 mg/kg cyclophosphamide (Cy) by a 1-hour intravenous infusion for 2 days was used in patients with acute and chronic myelogenous leukemia [20]. The remaining patients were conditioned with IV Cy (50 mg/kg/day) for 4 days in the alloidentical transplant patients and for 2 days in the unrelated donor transplant patients. This was followed by 300 CGy total body irradiation (TBI) daily for 4 days [21].

Determination of Percentage of Fragmented Erythrocytes

A single observer counted 500 red blood cells on blinded smears. The percentage of fragmented red cells was then calculated as previously described [8]. A frag-

TABLE I. Grading System for BMT-TTP*

Grade	LDH (U/L)	% Fragmented cells	Clinical BMT-TTP
0	Normal or increased	⩽1.2	Non
1	Normal	⩾1.3	Subclinical
2	Increased	1.3–4.8	Mild
3	Increased	4.9–9.6	Moderate
4	Increased	⩾9.7	Severe

*BMT, bone marrow transplant; TTP, thrombotic thrombocytopenic purpura; LDH, lactate dehydrogenase.

mented red blood cell was defined as a schistocyte (crescent, helmet, or triangle).

Definition of Thrombotic Thrombocytopenic Purpura

A thrombotic microangiopathy was defined by the presence of a peripheral blood smear showing increased fragmented blood cells (not explained by the presence of disseminated intravascular coagulation), an elevated lactate dehydrogenase level (LDH), and the presence of thrombocytopenia and/or a falling platelet count, except in the patients post-bone marrow transplantation, who had not had platelet recovery. Clinical parameters used to classify the thrombotic microangiopathy (except those post-bone marrow transplant) were as follows: (1) Fever >38°C, (2) neurologic dysfunction defined as any new abnormality in neuropsychiatric examination, (3) renal dysfunction defined as a serum creatinine (Cr) >1.5 mg/dl, and (4) platelet count <150 K/μl.

Grading System for Bone Marrow Transplant-Associated TTP

The TTP post transplantation was graded according to a grading system based upon LDH level and the percentage of fragmented erythrocytes as previously described [8]. This system was designed to reflect activity ranging from no evidence, to subclinical, to clinical thrombotic microangiopathy of varying severity (see Table I).

Statistics

The Kruskal-Wallis Anova by ranks test was used to compare the independent patient and control groups. Chi Square analysis was used to compare demographic features of the different groups. A P value < 0.05 was considered to be significant. Box range plots are used in Figures 1 and 2. In these plots, the median is indicated by a square point in the center of the box. The upper and lower limits of the box represent the 75 and 25% quartiles, respectively. These calculations were done using Statistica StatSoft 5.0 (Tulsa, OK).

Clinical Characteristics of Primary TTP and Secondary BMT-TTP Patients

Table II summarizes the clinical characteristics of the primary and secondary TTP patients, regarding sex, age, platelet count, lactate dehydrogenase level, creatinine, and presence of neurologic symptoms. Analysis was based upon the symptoms and clinical features present on the day of diagnosis. The ages were similar in all groups. Nineteen adult patients (4 males and 15 females), with a median age of 45 years (range 29–66 years) had primary TTP. Of the 47 adult patients (29 males and 18 females) with BMT-TTP, 14 had grade 2, 13 had grade 3, and 20 had grade 4 BMT-TTP. The median age in the grade 2 patient group was 41 years (range 25–55 years), for grade 3 was 42 years (range 40–45 years), and grade 4 was 32 years (range 28–43 years). There were more males in the BMT-TTP group (61%) as compared to the primary TTP group (26%), $P < 0.01$. The platelet counts were similar in all groups of patients.

The lactate dehydrogenase level (expressed as times the upper limit of normal) was elevated 5.6-fold in primary TTP (range 4.1–9.7) and 5.4-fold in grade 4 BMT-TTP (range 3.8–6.4). It was elevated to a lesser extent in grade 2 BMT-TTP (1.5-fold, range 0.8–2.5, $P < 0.001$) and grade 3 BMT-TTP (2.8-fold, range 2.1–3.7, $P = 0.01$).

Creatinine levels at diagnosis were higher in patients with primary TTP (median 1.6 mg/dl, range 1.2–6.6) and Grade 4 BMT-TTP (median 1.7 mg/dl, range 1.5–2.2, $P < 0.001$) as compared to grade 2 (median 1.1 mg/dl, range 0.8–1.7, $P < 0.001$.

Neurologic symptoms were more frequent in primary TTP (84%) and Grade 4 BMT-TTP (70%) as compared to Grade 2 BMT-TTP (14%) and Grade 3 BMT-TTP (54%), $P < 0.01$.

PAI-1 Antigen Levels in Controls and Patients With Primary TTP and Secondary BMT-TTP

Results for PAI-1 antigen levels (ng/ml) for controls (compared to the patients with primary TTP and secondary BMT-TTP) are shown as a box range plot in Figure 1. The limits of the boxes represent the 25 and 75% quartiles and the small central square shows the median.

The normal range for plasminogen activator inhibitor (PAI-1) is 0–41 ng/ml (mean ± 2SD). However, PAI-1 has a skewed distribution in the normal population. Therefore, the range expressed as 2.5 times the multiple of median is 0–20 ng/ml or expressed as quartiles is 5–24 ng/ml.

PAI-1 levels were elevated at diagnosis in patients with both primary TTP and secondary BMT-TTP as compared to controls, $P < 0.001$. Figure 1 shows the median value in primary TTP was 32 ng/ml (quartiles = 25–51 ng/ml). Furthermore, in the bone marrow transplant group, there was a wide range for PAI-1 (median = 44 ng/m with quartiles of 19–81 ng/ml).

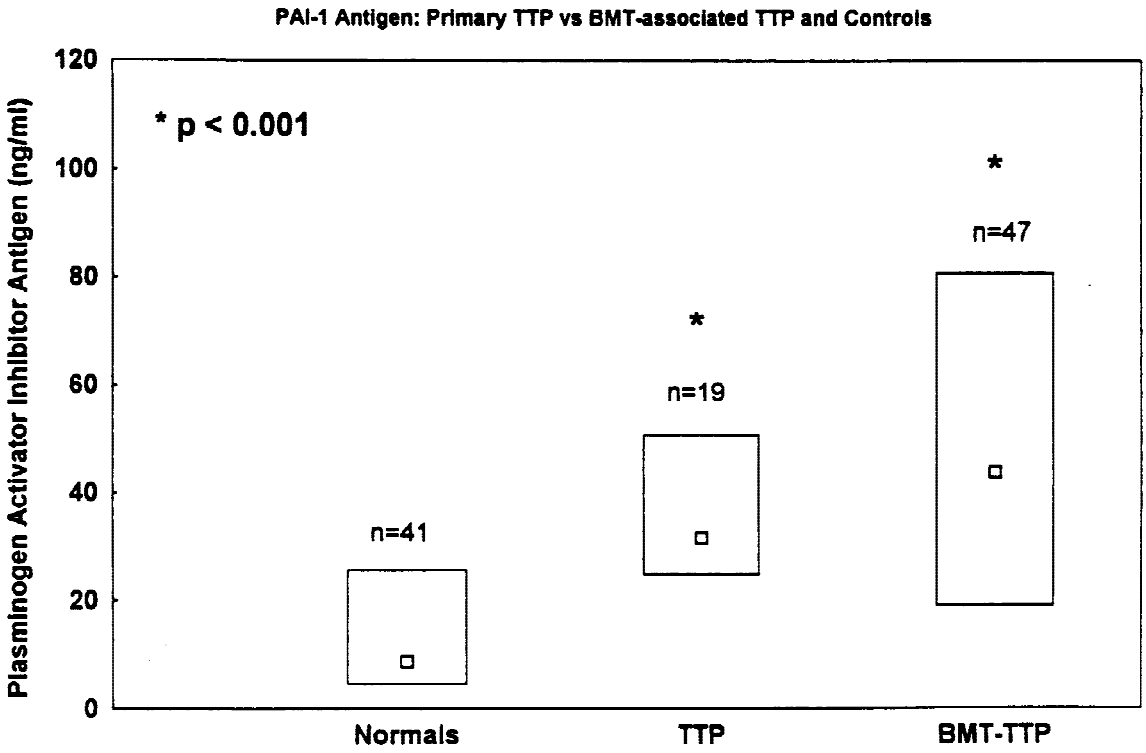

Fig. 1. Box range plots (median and quartiles) for plasminogen activator inhibitor antigen (PAI-1) levels in primary TTP and secondary TTP. n = number of patients.

PAI-1 Antigen Levels in Primary TTP Compared to the Different Grades (Grades 2–4) of Secondary BMT-TTP

Results for PAI-1 antigen levels (ng/ml) in patients with primary TTP compared to patients with different Grades (Grades 2–4) of BMT-TTP are also shown in box range plots in Figure 2.

Levels were lower in Grade 2 BMT-TTP (median = 16 ng/ml, quartiles 9–20) than in primary TTP. The values in Grade 2 BMT-TTP did not differ from normal. PAI-1 levels were elevated and similar in primary TTP (median = 32 ng/ml, quartiles 25–51) and Grade 3 BMT-TTP (median = 35 ng/ml, quartiles 19–48), $P = 0.7$. By contrast, levels in Grade 4 BMT-TTP were significantly higher than in Grade 3 BMT-TTP and primary TTP (median = 83 ng/ml, quartiles 60–143), $P < 0.001$.

DISCUSSION

Plasminogen activator inhibitor (PAI-1) is normally present in low concentrations in plasma, where it functions as an inhibitor of tissue plasminogen activator and urokinase [11,12]. High levels of PAI-1 have been reported in HUS [13–16] and in primary TTP [17] but have not been previously examined in BMT-TTP. Therefore, PAI-1 antigen levels were measured at diagnosis in a series of patients with primary and with secondary BMT-TTP. PAI-1 levels were elevated in both 1° TTP and in 2° BMT-TTP. However, the range of PAI-1 levels in BMT-TTP patients was broad (normal to being extremely elevated). PAI-1 antigen levels differed depending upon the Grade of BMT-TTP. Patients with Grade 2 BMT-TTP (usually a self-limited disorder) had normal levels. By contrast, PAI-1 levels were elevated and similar in patients with primary-TTP and those with moderate (Grade 3) BMT-TTP. The highest levels were seen in those patients with severe (Grade 4) BMT-TTP (usually a lethal disorder).

Primary TTP and BMT-TTP have considerable overlap in their clinical presentations. However, they have variable clinical courses. Adult TTP responds to plasma exchange [22]. BMT-TTP generally does not improve with plasma exchange with plasma infusion [8–10]. Grade 2 BMT-TTP is usually self-limited [8]; whereas, patients with untreated primary TTP usually die. Grades 3 and 4 BMT-TTP are often multifactorial [7] (associated with GVHD, CMV, or fungal infections) and are often lethal (in spite of plasma exchange) and result in multiorgan failure [7–10].

In adult TTP, the pathogenic lesion is the formation of von Willebrand rich platelet microthrombi with a small amount of fibrin [23]. The persistence of platelet plugs with associated fibrin led Kwaan and colleagues [24,25] to postulate that hypofibrinolysis may be contributory to

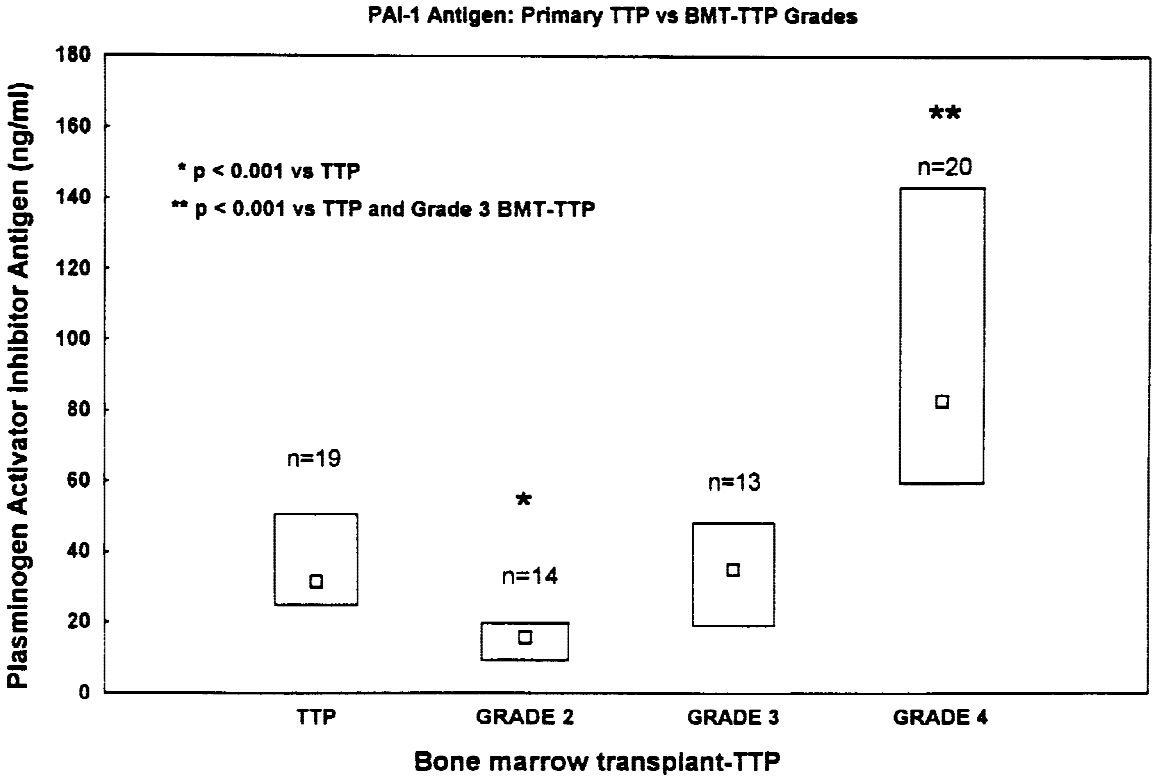

Fig. 2. Box range plots (median and quartiles) for plasminogen activator inhibitor antigen (PAI-1) levels in primary TTP vs. different grades of secondary BMT-TTP. n = number of patients.

TABLE II. Clinical Characteristics of Primary TTP and BMT-TTP Patients at Diagnosis*

Syndrome	M:F	Age (years)	Platelets (K/μl)	LD × upper normal	Creatinine (CR) (mg/dl)	Neurologic symptoms
Normal range			150–400	—	(0.5–1.1)	
TTP	4:15	45	18	5.6	1.6	16/19
		(29–66)	(10–41)	(4.1–9.7)	(1.2–6.6)	(84%)
BMT-TM	9:5	41	30	1.5	1.1	2/14
Grade 2		(25–55)	(20–39)	(0.8–2.5)	(0.8–1.7)	(14%)
BMT-TM	5:8	42	20	2.8	1.3	7/13
Grade 3		(40–45)	(20–20)	(2.1–3.7)	(1.1–1.7)	(54%)
BMT-TM	15:5	32	< 20[a]	5.4	1.7	14/20
Grade 4		(28–43)		(3.8–6.4)	(1.5–2.2)	(70%)

*Data shown as median and quartiles in parentheses. TTP, thrombotic thrombocytopenic purpura; BMT, bone marrow transplant; LD, lactic dehydrogenase.

[a]All patients receiving platelet transfusions were considered to have platelet counts < 20 K/μl.

TTP. Kwaan et al. [24,25] have reported that tissue plasminogen activator (tPA) activity is absent from the occluded vessels of patients with TTP but present in uninvolved vessels. By contrast, the vessels of patients with intravascular thrombosis due to conditions other than TTP had a normal tPA staining pattern. Plasma fibrinolytic activity was normal in the TTP patients. One study has indicated that tPA and PAI-1 levels are proportional in TTP [17]. The present study did not measure tPA or functional activity of PAI-1. Therefore, the impact on fibrinolysis is uncertain.

HUS patients have been reported to have lowered levels of plasminogen activating activity and the presence of plasma activity that is inhibitory to plasminogen activation [16]. Recently PAI-1 (using both antigenic [13–16] and functional assays [13]) has been found to be elevated in HUS. In addition, PAI-1 levels have been shown to correlate with a poor outcome in HUS [13,14]. The source of the elevated PAI-1 is unknown.

These results clearly show that PAI-1 levels are elevated in primary TTP and BMT-TTP. Furthermore, the severity of BMT-TTP appears to correlate with PAI-1. It

is reasonable to postulate that raised levels of PAI-1 would inhibit tPA-mediated plasmin generation and promote fibrin deposition on platelet microthrombi, thus exacerbating the hemolysis and vascular damage. Direct proof of this hypothesis requires further investigation.

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